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A Kind of Gene Chip Carrier for Multi-person Diagnosis

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Claims

1. This invention involves a kind of gene chip carrier for disease diagnosis. The characteristic of the carrier is that there are multiple evenly distributed spotting pools in the spotting zone. Each spotting pool is 1mm-3mm long, 1mm-3mm wide, and 1mm-3mm deep.

2. According to the gene chip carrier as described in Item 1 of the Claims, the said gene chip carrier consists of two parts. The first part is a non-spotting zone undergone frosting treatment, while the second part is a spotting zone. In addition:

(1) The said non-spotting zone may be located on one side or two sides of the chip with the total width of 10-25mm;

(2) The said spotting zone consists of multiple evenly distributed spotting pool.

3. According to the gene chip carrier as described in Item 1 and Item 2 of the Claims, the material of the said gene chip carrier can be glass, plastic, or ceramic.

4. According to the gene chip carrier as described in Item 1

and Item 2 of the Claims, each response cell and the response surface parallel to the carrier surface are undergone special treatment. The response surface has amino or mercapto that can form electron pair bond with DNA.

5. According to the gene chip carrier as described in Item 1 and Item 2 of the Claims, the gene chip on each response cell surface consists of two parts. The first part is detection and monitoring system while the second part is disease diagnosis system.

6. According to the gene chip carrier as described in Item 1 and Item 2 of the Claims, the carrier can be used for multi-person, multi-disease diagnosis.

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Instructions

A Kind of Gene Chip Carrier for Multi-person Diagnosis

This invention concerns a kind of gene chips in the field of gene technology. Specifically, the invention concerns a kind of gene chip carrier for multi-person, multi-disease diagnosis.

Gene chip is a cutting-edge biological technology developed since 1990s. With this technology, a great number of target gene segments are arranged orderly and intensively (the spacing between points is usually less than 500μm) on carriers such as glass and silicon. Such a carrier is called gene chip. As far as the manufacturing method is concerned, gene chips can be classified into two categories: in situ synthesis method (DNAchip) and micro array method (DNA microarray). The DNAchip method was invented by Fodor et al. from Affymetrix Inc. Fodor et al. applied optical etching technique commonly seen in semiconductor industry in the DNA synthesis chemistry, and used single nucleotide or other biological macromolecules as the substrate to synthesize in situ oligonucleotide on a glass crystal plate. In each synthesis cycle, specific amount of

[1] Numbers in the margin indicate pagination in the foreign text.

nucleotide is bonded onto the plate. The process continues until the set oligonucleotide length is reached. Each oligonucleotide segment represents a special gene that exists on a specific position of the DNA chip. The DNA microarray method was first invented by P. Brown et al. from Stanford University. With this method, cDNA obtained with PCR method is directly arranged through needling or spraying on the media such as glass plate to make gene chips.

Fluorescent detection and computer software can be applied on gene chips for data comparison and analysis, thus achieving the purpose of analyzing biological information fast, effectively, and in large volume.

The concept of gene chip can be traced back to southern blot hybridization, which is to form a complementary chain by way of basic group pairing system. Afterwards, the northern blot hybridization technique and point hybridization technique were developed. In all of the three techniques, the nucleic acid spotting was fixed on the filter film. Since the spotting can easily disperse, the spotting density on unit area is limited, making it impossible to conduct large-scale, high volume DNA hybridization. In the meantime, the large filter film area requires more probes, which lowers the detection sensitivity. In order to enhance the spotting density and detection sensitivity

while reducing the amount of probes, the gene chip technique featuring glass and silicon materials as the carrier was gradually developed.

The U.S. Affymetrix Inc. led the research in this area in late 1980s and early 1990s (Fodor, S.P.A. et al.) (1991) Science 251, 767-773). In 1992, the company employed semiconductor photographing plate technique to have synthesized in situ an oligopolynucleotide segment on a glass plate of about 1cm2 area, giving birth to the world's first gene chip (Southern, E., Maskos, U. & Elder, R. (1992) Genomics 13, 1008-1017). In the same time, the probe fluorescent mark technique, the laser co-focal scanning (U.S. Patent 5981965) technique and computer analysis (U.S. Patent 5974164) technique were also developed accordingly. In 1995, the first (microarray) gene chip with glass as the carrier was developed at Stanford University in the U.S.A. (Schena, M. et al. (1995) Science 270 (20): 467-480), symbolizing that the gene chip technology has stepped in extensive research and application period.

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The in situ synthesized oligopolynucleotide chip has the advantages of high intensity and ability to synthesize oligopolynucleotide of any sequences, which is suitable for DNA sequence detection and SNP analysis. The weakness, however, is

that the length of the synthesized polyoligonucleotide is limited, leading to poor gene specificity and high rate of synthesis errors as the length increases. This kind of chip is much less desirable than cDNA chip in the research on gene expression spectrum is concerned. In the cDNA chip, micro quantity cDNA segments are intensively arranged in a matrix form and then solidified, thus forming a microarray (DeRisi J, et al. (1997) Science 278, 680-686). Although the gene spotting density of the cDNA chip is not as high as that of in situ synthesized oligopolynucleotide chip, it is much higher than that of traditional carriers such as mixed fiber filter film or nylon film. In fact, the density of cDNA chip can reach 40,000 genes per carrier glass plate. The most remarkable advantage of cDNA chip is that its target gene detection specificity is very good, which makes the expression spectrum research result very reliable. Today, many national laboratories and major pharmaceutical companies have endorsed this kind of chips (Baldwin D. et al. (1999) Curr. Opin. Plant Biol. 2 (2): 96-103).

Gene chips was first developed for the high-volume and large-scale research on gene functions. However, with the maturity of gene chip technology, people discovered, with pleasant surprise, that gene chip has a unique application prospect and huge commercial market in the aspect of contagious

disease and hereditary disease diagnosis. For example, in an infectious pathogen diagnosis chip, the characteristic gene segment (target gene) to be tested is fixed on a glass plate. The DNA or RNA extracted from the blood serum of a patient is augmented, marked with fluorescent light, and undergone hybridization with the chip. The hybridization signal is scanned by a scanner and then analyzed by computer to determine the negative or positive outcome. The diagnosis chip is superior to other detection measures in that:

(1) The detection spotting can be segments of various kinds of pathogenic genes, thus enhancing the detection efficiency;

(2) The organism immunity reaction becomes unnecessary, offering early diagnosis and small consumption of spotting to be detected;

(3) The diagnosis chip technique is a molecular diagnosis method combining DNA hybridization technique and fluorescent marking technique, featuring extremely high sensitivity, specificity, and reliability;

(4) Diagnosis chip features high automation extent that is favorable for extensive popularization.

In the field of disease diagnosis, some single disease gene diagnosis chips have been made available in the market. For example, Affymetrix Inc. has made use of gene chips in the

research on virus of AIDS (HIV) and has put commercial GeneChip HIV PRT diagnosis chip on market for early diagnosis of AIDS.

However, the application of chip technology on disease diagnosis is still in the starting stage due to the following reasons:

(1) Gene chip technique is a brand new technique that has very high demand on qualified personnel, technology, and capital investment. Today, only few research institutes and high technology companies have a comprehensive appreciation of this technology.

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(2) If one chip is only capable of conducting single-person diagnosis on one or several potential diseases, then the cost for chip detection will be high and the detection efficiency will be low. Consequently, the advantages of gene chips on high-volume and large-scale detection will not be fully utilized. For this reason, many domestic and foreign institutions are dedicated to the research and development of multi-person gene chip carriers in order to make full use of technical features and advantages of gene chips for practical applications of clinic diagnosis.

The purpose of this invention is to provide a kind of gene chip carriers for multi-person diagnosis. Multiple independent response cells exist on one chip with each response cell serving

as an independent response cell that includes detection and monitoring system and disease diagnosis system. Each independent response cell can be used to conduct single person detection and reflects the health status of the individual. As such, one chip is able to provide diagnosis to the health status of multiple persons at the same time.

The concept of the invention is as follows:

(1) In this invention, multiple independent response cells exist on one gene chip. Each independent response cell constitutes a single-person health status detection system and can be used to check the health status of one individual. One chip is able to provide diagnosis to the health status of multiple persons at the same time;

(2) In order to form independent response cell and avoid cross contamination, concave independent response cells are designed on the gene chip carrier surface.

The invention is realized in the following ways:

In order for the diagnosis chip to meet the multi-disease, multi-person diagnosis requirement, we conduct the following design to the gene chip carrier:

The said gene chip carrier material can be glass, plastic, or ceramic. The gene chip carrier surface consists of two parts: the non-spotting zone and the spotting zone.

(1) One gene chip has one to two non-spotting zones. If there is one non-spotting zone, then the spotting zone is located on one side of the chip with the width of 10~25mm. If there are two non-spotting zones, then the non-spotting zones are located on both sides of the chip with the width of 5~10mm each. The surfaces of the non-spotting zones are undergone frosted treatment. The non-spotting zone is used for operation contact during chip detection.

(2) The other part of the chip is spotting zone, in which there are a number of spotting pools forming independent response cells. Each spotting pool is 1mm~6mm long, 1mm~8mm wide, and 1mm~3mm deep. Concave square and rectangular response cells are formed on the carrier surface. The actual area of each response cell has something to do with the spotting number. The response cells are evenly distributed in the non-spotting zone. Each response cell and the response surface parallel to the carrier surface are undergone special treatment. The response surface has amino or mercapto that can form electron pair bond with DNA. The surface treatment technique is a known technique.

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(3) The gene chip in each response cell consists of two parts. The first part is a detection and monitoring system, while

the second part is a disease diagnosis system.

The detection and monitoring system is comprised of at least four control elements, which are respectively:

1. Spotting solution, which is a negative control element (1). If no hybridization signal is detected, it shows that the spotting solution is normal. If hybridization signal is detected, it shows that the spotting solution is contaminated;

2. Plant gene a, which is a negative control element (2). If no hybridization signal is detected, it shows that the spotting solution is normal. If hybridization signal is detected, it shows that the system is contaminated;

If hybridization signal shows up in either case of 1 or 2, the chip cannot be further used.

3. Plant gene b, which is a positive control element (1). While extracting blood RNA, the RNA of the plant gene is added proportionally to the blood serum sample to be tested, and is undergone reverse transcript with the flow of experiment. If hybridization signal is detected in the sample after hybridization, it means that the system is normal. If no hybridization signal is detected, it shows that the RNA extraction process is problematic or the reverse transcript is abnormal;

4. Plant gene c, which is a positive control element (2).

During the process of PCR augmentation and mixing of fluorescent base substance, the DNA of the plant gene is added proportionally to the product of reverse transcript in order to detect the mixing efficiency of the fluorescent base substance. If hybridization signal is detected in the sample after hybridization, it means that the system is normal. If no hybridization signal is detected, it shows that the marking effect is less desirable.

The said spotting solution is a buffer solution containing ion polymers. The spotting solution named Micro-Spotting Solution made by the U.S. Telechem Corporation can be used.

The gene sequence of the said plant gene a is shown in the following plant gene a – Sequence a:

Plant a – Sequence a:

Arabidopsis thaliana (*Ni Nan Qi*) SUPERMAN (sup) gene

AATTGCCAACAGGATCATGATTATCTTCTAGGGTTTTCATGGCCACCAAGATCCTACACT
TGCAGCTTCTGCAAAAGGGAATTCAGATCGGCTCAAGCACTTGGTGGCCACATGAATGT
TCACAGAAGAGACAGAGCAAGACTCAGATTACAACAGTCTCCATCATCATCTTCAACACC
TTCTCCTCCTTACCCTAA-CCCTAATTACTCTTACTCAACCATGGCAAACTCTCCTCCT
CCTCATCATTCTCCTCTAACCCTATTTCCAACCCTTTCTCCTCCATCCTCACCAAGATAT
AGGGCAGGTTTGATCCGTTCCTTGAGCCCCAAGTCAAAACATACACCAGAAAACGCTTGT
AAGACTAAGAAATCATCTCTTTTAGTGGAGGCTGGAGAGGCTACAAGGTTCACCAGTAAA
GATGCTTGCAAGATCCTGAGGAATGATGAAATCATCAGCTTGGAGCTTGAGATTGGTTTC
ATTAACGAATCAGAGCAAGATCTGGATCTAGAACTCCGTTTGGGTTTCGCTTAATTAGAT
GGTAATAACTTTATCCATAAAGGATTCG--AAGTTCACAATTCTAGAAGATATGATGCTT
CTCTAAGGTTAATTAGTTTCATCCATATGAAATTCTCTAAGCTTGCTATTTAGTAGAACG

The plant gene a can be obtained by cloning the arabidopsis thaliana SUPERMAN (sup) gene, which is a known technique.

The gene sequence of the said plant gene b is shown in the following plant gene b – Sequence b:

Plant b – Sequence b (the shaded part refers to PCR augmentation reference sequence):

Rice Usn 5sRNA gene

TAATACGAGCACTATAGGGAGAGGCGAATTGGGTACCGGGCCCCCCCTCGAGAGCGAGCTCAATGGC
TACAGGACCAACCTTATCAAAGAGAGCATCAGAATGGGTTACAATGACATTGGTGANGGCTTCTATG
CTCATGGCCACCTTTCAGATGCCTTCAAAAGCTACATCCGTACACGTGATTATTGTACCACTTCCAA
GCATATAGTTCAGATGTGTATGAATGTGATTCTGGTTAGCATTCAGCTTGGGAC

The plant gene b can be obtained by cloning the rice Usn 5sRNA gene, which is a known technique.

The gene sequence of the said plant gene c is shown in the following plant gene c – Sequence c:

Plant c – Sequence c (the shaded part refers to PCR augmentation reference sequence):

Rice Usn 5sRNA gene

ATAAACCGTCAGTAAGGGAACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTG

GATCCCCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATNNCGTCGACCTCGAGTTTTTTTTTTT

TTTTTTTACTAAGAGTTACTATGCATCACAGTACCAGCTCTAAGCATTTATATTTTAAAAAATAGCT

TGGCCTCAAATGTGACAGATCGTTCTGTCAAGTCTCTAGATGCTTAAATATCCAGAGCTGTTTACAC

AGCATCATATTTAAAACCACAGGAAGCAAGGAATTCGTCCGA

The plant gene c can be obtained by cloning the rice Usn 5sRNA gene, which is a known technique.

The disease diagnosis system in each response cell fixes the conserved area of several pathogens on the chip as target genes, which, coupled with corresponding target gene augmentation reference substance and chip diagnosis techniques such as DNA hybridization and fluorescent light marking techniques, can be used for diagnosis of clinical diseases. The chip diagnosis technique is a known technique. Next, we further explain the details of the inventions by way of the following example. However, the example does not restrict the scope of protection to the invention. Technical personnel concerned may fabricate other types of multi-person, multi-disease diagnosis chips based on the concept of the invention and the published technical schemes, which are also within the range of protection to the invention.

Fig. 1 is the diagram of one gene chip carrier for multi-person diagnosis based on the invention.

Fig. 2 is the diagram of another gene chip carrier for multi-person diagnosis based on the invention.

Fig. 3 is the amplified diagram of three kinds of response pools based on the invention.

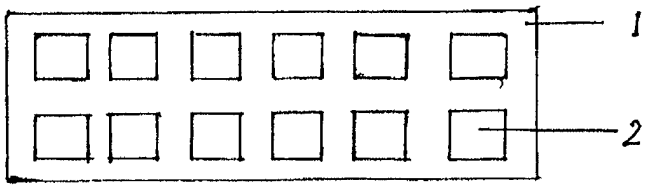

图 1

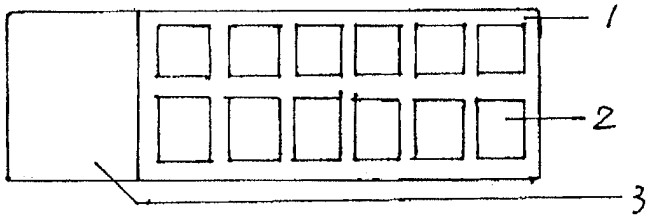

图 2

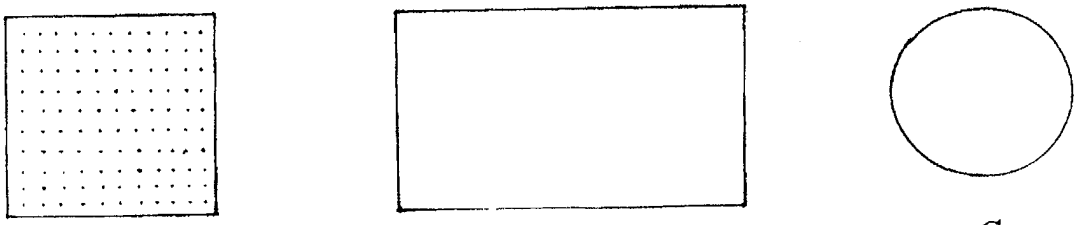

图 3

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[54]发明名称 一种用于多人份诊断的基因芯片载体

[57]摘要

本发明公开了一种疾病诊断用基因芯片载体，其特征在于点样区内有多个呈均匀分布的点样池，其长度为1mm－3mm，宽度为1mm－3mm，深度为1mm－3mm，每个点样池下凹的载体表面构成一个独立的反应单元。每一反应单元构成一个单人份健康状况检测系统，可对一个被检个体的健康状况进行检测，一块芯片就能同时进行多疾病、多人份的诊断而不会产生交叉污染。

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权　利　要　求　书

1. 一种疾病诊断用基因芯片载体，其特征在于点样区内有多个呈均匀分布的点样池，其长度为 1mm-3mm，宽度为 1mm－3mm，深度为 1mm－3mm，每个点样池下凹的载体表面构成一个独立的反应单元。

2. 如权利要求 1 所述的基因芯片载体，其特征在于所说的基因芯片载体由两个部分组成，第一部分为经表面经磨沙处理过的非点样区，第二部分为点样区，而且：

（1）所述非点样区可只位于芯片的一侧或分列于两侧，其总宽度为 10-25mm；

（2）所述的点样区内是多个呈均匀分布的点样池。

3. 如权利要求 1 或 2 所述的基因芯片载体，其特征在于所述基因芯片载体的材料可以是玻璃，塑料或陶瓷。

4. 如权利要求 1 或 2 所述的基因芯片载体，其特征在于，每个反应单元与载体承载面平行的反应表面经特殊处理，表面具有与 DNA 形成共价键的氨基或巯基。

5. 如权利要求 1 或 2 所述的基因芯片载体，其特征在于，每一反应单元表面基因芯片包括两个部分组成，第一部分为检测监控系统，第二部分为疾病诊断系统。

6. 如权利要求 1 或 2 所述的基因芯片载体的用途，其特征在于用于多人份、多疾病诊断。

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说　　明　　书

一种用于多人份诊断的基因芯片载体

本发明属于基因技术领域，涉及一种基因芯片，尤其涉及一种用于多人份，多疾病诊断的基因芯片载体。

基因芯片是二十世纪九十年代发展起来的一项前沿生物技术。将大量的靶基因片段有序地、高密度地（点与点之间的距离一般小于500μm）排列在玻璃、硅等载体上，称之为基因芯片。基因芯片从制作上可分为两大类：原位合成法（DNAchip）和微矩阵法（DNA mircoarray），原位合成法由Affymetrix公司的Fodor等发明，他们将半导体中的光蚀刻技术运用到DNA合成化学中，用单核苷酸或其它生物大分子为底物，在玻璃晶片上原位合成寡核苷酸，每次循环都有特定的核苷酸结合上去，直到设定的寡核苷酸长度，每个寡核苷酸片段代表了一种特定的基因，存在于DNA芯片的特定位置上。微矩阵法最早由Stanford大学的P. Brown等发明，将PCR等方法得到的cDNA用针点或喷点的方法直接排列到玻片等介质上，从而制备成芯片。

基因芯片可以用荧光检测和计算机软件进行数据的比较和分析，以达到快速、高效、高通量地分析生物信息的目的。

基因芯片的概念可追溯到southern blot杂交技术，即DNA-DNA之间通过碱基配对机制形成互补链。随后又发展出northern blot杂交和点杂交技术。这三种技术都是将核酸样品固定在滤膜上，样品容易扩散，因此在单位面积上点样的密度受到限制，无法进行大规模、高通量的DNA杂交。同时，由于滤膜面积较大而需较多探针量，检测灵敏度较低。为了提高点样密度和检测灵敏度，降低探针用量，以玻璃、硅等材料为载体的基因芯片技术逐步得到了发展。

美国affymetrix公司于二十世纪八十年代末至90年代初，率先开展了这方面的研究(Fodor, S. P. A. et al. （1991）Science 251, 767-773.)。1992年，该公司运用半导体照相平板技术，在1cm^{2}左右的玻片上原位合成寡聚核苷酸片段，诞生了世界上第一块基因芯片(Southern, E., Maskos, U. & Elder, R. （1992）Genomics 13, 1008-1017.)。同时，探针的荧光标记，激光共聚焦扫描(U. S Patent 5981965)和计算机分析(U. S Patent 5974164)等技术也随之发展。 1995年，第一块以玻璃为载体的基因芯片（微矩阵）在美国Stanford大学诞生(Schena, M. 等

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（1995）Science. 270.（20）：467-480.），这标志着基因芯片技术步入了广泛研究和应用的时期。

原位合成寡聚核苷酸芯片具有密集程度高，可合成任意序列的寡聚核苷酸等优点，适用于DNA序列测定、SNP分析等。但其缺点是合成寡聚核苷酸长度有限，因而基因特异性差，而且随长度的增加合成错误率随之增高，作为基因表达谱研究远不如cDNA芯片。cDNA芯片是将微量cDNA片段在玻璃等载体上按矩阵密集排列并固化，也叫微矩阵(Microarray) (DeRisi, J.等（1997）Science 278, 680-686.)。基因点样密度虽不及原位合成寡聚核苷酸芯片高，但比用传统载体，如混合纤维素滤膜或尼龙膜的点样密度要高得多，可达到每张载玻片4万个基因。而cDNA芯片最大的优点是靶基因检测特异性非常好，用作表达谱研究结果可靠。目前许多国家实验室和大制药公司都用此类芯片(Baldwin, D等 （1999）Curr Opin Plant Biol 2（2）:96-103.)。

基因芯片最早是由于高通量、大规模研究基因功能的需求而产生的，但随着基因芯片技术的日渐成熟，人们惊喜地发现基因芯片在传染性疾病和遗传病的诊断上有独特的应用前景和巨大的商业市场。感染性病原体诊断芯片就是将待测病原体的特征基因片段（靶基因）固定于玻片上制成芯片，将从病人血清中抽提出病原体的DNA或RNA经扩增标记萤光后与芯片进行杂交，杂交信号由扫描仪扫描，再经计算机分析，判断阴阳性。诊断芯片区别于其他检测手段的优越性在于：

（1）检测样品为各类致病基因片段，提高了检测效率；

（2）因无需机体免疫反应，能及早诊断，且待测样品用量较少；

（3）诊断芯片技术是DNA杂交技术和荧光标记技术相结合的分子诊断方法，有极高的灵敏度、特异性和可靠性；

（4）自动化程度高，利于大规模推广应用。

在疾病诊断方面，已有一些单一疾病基因诊断芯片产品上市，例如美国的Affymetrix 公司已利用基因芯片进行爱滋病病毒（HIV）的研究工作，并有商业化的 GeneChip HIV PRT 诊断芯片上市，用于爱滋病的早期诊断。

但芯片技术在疾病诊断上的应用尚处于起步阶段，还未大面积推广，原因主要有：（1）基因芯片技术是一项全新的技术，对人才、技术、资金的要求很高，目前只有一部分的科研机构和高科技公司完全掌握了该项技术；

（2）如果一种芯片只能进行单人份检测，诊断一个被检者可能存在的一种

疾病或几种疾病，芯片检测成本较贵，检测效率不高，未充分发挥基因芯片可进行高通量、大规模检测的优势和特点。因此，目前国内外已有多家机构致力于研究开发可用于多人份的基因芯片载体，以真正实现利用基因芯片的技术特征和优势应用于临床诊断的实际应用。

本发明的目的在于提供一种进行多人份诊断的基因芯片载体，在一块芯片上同时存在多个独立的反应单元，各反应单元是一个独立的诊断单元，包括检测监控系统和疾病诊断系统；每一独立反应单元可进行单人份检测，反映一个被检个体的健康状况，所以，一块芯片就能够同时诊断多人份的健康状况，因此一块芯片就能进行多疾病、多人份的诊断。

发明的构思是这样的：

（1）本发明的一块基因芯片上同时具有多个独立的反应单元，每一反应单元构成一个单人份健康状况检测系统，可对一个被检个体的健康状况进行检测，一块芯片就能同时进行多疾病、多人份的诊断；

（2）为形成独立反应单元和避免交叉污染，可在基因芯片载体表面设计下凹的独立反应单元；

本发明亦是这样实现的：

为使诊断芯片符合多疾病、多人份诊断的要求，我们对基因芯片载体进行如下设计：

所说的基因芯片载体材料可以为玻璃、塑料以及 陶瓷 ，基因芯片载体表面由两个部分组成，第一部分为非点样区，第二部分为点样区。

（1）一块基因芯片有 一 到两个 非点样区， 如 果有一个非点样区，该点样区位于芯片的一侧，宽度为 10~25mm 如果有两个非点样区，该非点样区分别位于芯片的两侧，宽度为 5~10mm。非点样区表面经磨沙处理，非点样区用于芯片检测时的操作接触。

（2）芯片其他部分为点样区，点样区内有若干数量的点样池，形成独立的反应单元。每个点样池为长度为 1mm~6mm, 宽度为 1mm~8mm，深度为 1mm—3mm，在载体表面形成下凹的正方形和长方形反应单元，每个反应单元的实际面积大小与所点点数的多少有关。反应单元在非点样区呈均匀分布。每个反应单元与载体承载面平行的反应表面经特殊处理，表面具有与 DNA 形成共价键的氨基、巯基等。表面处理为已有技术。

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疾病或几种疾病，芯片检测成本较贵，检测效率不高，未充分发挥基因芯片可进行高通量、大规模检测的优势和特点。因此，目前国内外已有多家机构致力于研究开发可用于多人份的基因芯片载体，以真正实现利用基因芯片的技术特征和优势应用于临床诊断的实际应用。

本发明的目的在于提供一种进行多人份诊断的基因芯片载体，在一块芯片上同时存在多个独立的反应单元，各反应单元是一个独立的诊断单元，包括检测监控系统和疾病诊断系统；每一独立反应单元可进行单人份检测，反映一个被检个体的健康状况，所以，一块芯片就能够同时诊断多人份的健康状况，因此一块芯片就能进行多疾病、多人份的诊断。

发明的构思是这样的：

（1）本发明的一块基因芯片上同时具有多个独立的反应单元，每一反应单元构成一个单人份健康状况检测系统，可对一个被检个体的健康状况进行检测，一块芯片就能同时进行多疾病、多人份的诊断；

（2）为形成独立反应单元和避免交叉污染，可在基因芯片载体表面设计下凹的独立反应单元；

本发明亦是这样实现的：

为使诊断芯片符合多疾病、多人份诊断的要求，我们对基因芯片载体进行如下设计：

所说的基因芯片载体材料可以为玻璃、塑料以及 陶瓷 ，基因芯片载体表面由两个部分组成，第一部分为非点样区，第二部分为点样区。

（1）一块基因芯片有 一 到两个 非点样区， 如 果有一个非点样区，该点样区位于芯片的一侧，宽度为 10~25mm 如果有两个非点样区，该非点样区分别位于芯片的两侧，宽度为 5~10mm。非点样区表面经磨沙处理，非点样区用于芯片检测时的操作接触。

（2）芯片其他部分为点样区，点样区内有若干数量的点样池，形成独立的反应单元。每个点样池为长度为 1mm~6mm, 宽度为 1mm~8mm，深度为 1mm—3mm，在载体表面形成下凹的正方形和长方形反应单元，每个反应单元的实际面积大小与所点点数的多少有关。反应单元在非点样区呈均匀分布。每个反应单元与载体承载面平行的反应表面经特殊处理，表面具有与 DNA 形成共价键的氨基、巯基等。表面处理为已有技术。

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（3）每一反应单元内基因芯片包括两个部分组成，第一部分为检测监控系统，第二部分为疾病诊断系统。.

检测监控系统至少有四个对照元素构成，分别为：

1. 点样液，为阴性对照（1），若杂交后检测无信号，表明正常；若有杂交信号，表明点样液有污染；

2. 为植物基因a，为阴性对照（2），若杂交后检测无信号，表明正常；若有杂交信号表明系统有污染；

1或2其中之一出现杂交信号，该芯片不能使用；

3. 为植物基因b，为阳性对照（1），该植物基因的RNA在抽提血RNA时按一定的比例加入到待检的血清样品中，并随着实验流程进行逆转录。若杂交后该样品有杂交信号为正常；若无杂交信号，表明RNA抽提过程有问题或逆转录不正常；

4. 为植物基因c，为阳性对照（2），该植物基因DNA在PCR扩增掺入荧光底物时按一定的比例加入到逆转录的产物中，以检测荧光底物的掺入效率。若杂交后该样品有杂交信号为正常；若无杂交信号，表明标记效果不理想。

所说的点样液为一种包含离子多聚物的缓冲液，可以采用美国Telechem公司生产的、商品名为Micro-Spotting Solution的点样液；

所说的为植物基因a的基因序列如下述的植物基因a Sequence a所示：

植物基因a Sequence a：

Arabidopsis thaliana（拟南芥） SUPERMAN (sup) gene

AATTGCCAACAGGATCATGATTATCTTCTAGGGTTTTCATGGCCACCAAGATCCTACACT

TGCAGCTTCTGCAAAAGGGAATTCAGATCGGCTCAAGCACTTGGTGGCCACATGAATGT

TCACAGAAGAGACAGAGCAAGACTCAGATTACAACAGTCTCCATCATCATCTTCAACACC

TTCTCCTCCTTACCCTAA-CCCTAATTACTCTTACTCAACCATGGCAAACTCTCCTCCT

CCTCATCATTCTCCTCTAACCCTATTTCCAACCCTTTCTCCTCCATCCTCACCAAGATAT

AGGGCAGGTTTGATCCGTTCCTTGAGCCCCAAGTCAAAACATACACCAGAAAACGCTTGT

AAGACTAAGAAATCATCTCTTTTAGTGGAGGCTGGAGAGGCTACAAGGTTCACCAGTAAA

GATGCTTGCAAGATCCTGAGGAATGATGAAATCATCAGCTTGGAGCTTGAGATTGGTTTG

ATTAACGAATCAGAGCAAGATCTGGATCTAGAACTCCGTTTGGGTTTCGCTTAATTAGAT

GGTAATAACTTTATCCATAAAGGATTCG--AAGTTCACAATTCTAGAAGATATGATGCTT

CTCTAAGGTTAATTAGTTTCATCCATATGAAATTCTCTAAGCTTGCTATTTAGTAGAACG

该植物基因a可通过对拟南芥 SUPERMAN (sup) gene克隆获得，此为已有技术。

所说的为植物基因b的基因序列如下述的植物基因b Sequence b所示：

植物基因b Sequence b（阴影部分为其PCR扩增引物序列）：

水稻 Usn 5sRNA gene

TAATACGACCACTATAGGGAGAGGCGAATTGGGTACCGGGCCCCCCCTCGAGAGCGAGCTCAATGGC

TACAGGACCAACCTTATCAAAGAGAGCATCAGAATGGGTTACAATGACATTGGTGANGGCTTCTATG

CTCATGGCCACCTTTCAGATGCCTTCAAAAGCTACATCCGTACACGTGATTATTGTACCACTTCCAA

GCATATAGTTCAGATGTGTATGAATGTGATTCTGGTTAGCATTGAGTTGCCAC

该植物基因b可通过对水稻 Usn 5sRNA gene克隆获得，此为已有技术。

所说的为植物基因c的基因序列如下述的植物基因c Sequence c所示：

植物基因c Sequence c（阴影部分为其PCR扩增引物序列）：

水稻 Usn 5sRNA gene

ATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCCACCGCGGTGGCGGCCGCTCTAGAACTAGTG

GATCCCCCGGGCTGCAGGAATTCGATATCAAGCTTATCGATNNCGTCGACCTCGAGTTTTTTTTTTT

TTTTTTTACTAAGAGTTACTATGCATCACAGTACCAGCTCTAAGCATTTATATTTTAAAAAATAGCT

TGGCCTCAAATGTGACAGATCGTTCTGTCAAGTCTCTAGATGCTTAAATATCCAGAGCTGTTTACAC

AGCATCATATTTAAAACCACAGCAAGCAAGGAATTGTCCGA

该植物基因c可通过对水稻 Usn 5sRNA gene克隆获得，此为已有技术。

每一反应单元的疾病诊断系统将若干种病原体的保守区作为靶基因固定在芯片上，配以相应的靶基因扩增引物，并通过 DNA 杂交、荧光标记等芯片诊断技术，进行临床疾病的诊断。芯片诊断技术为已有技术。

以下将通过实施例对本发明的有关细节作进一步的阐述，但实施例并不限制本发明的保护范围，有关的技术人员完全可以根据本发明的构思和所公开的技术方案，举一反三地制备其它类型的多人份、多疾病诊断芯片，这也在本发明的保护范围之内。

图 1 是本发明的一个多人份诊断的基因芯片载体的示意图；

图 2 是本发明的另一个多人份诊断的基因芯片载体的示意图；

图 3 所示的是本发明的三种不同反应池的放大示意图。

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说　明　书　附　图

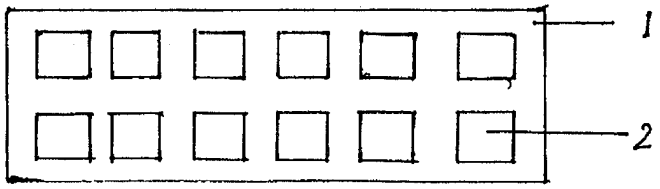

图 1

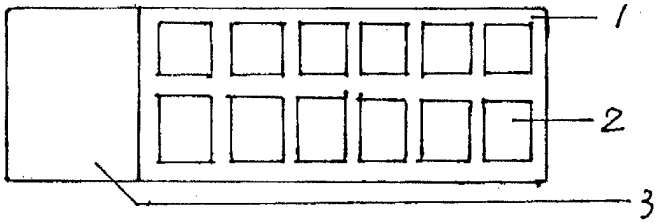

图 2

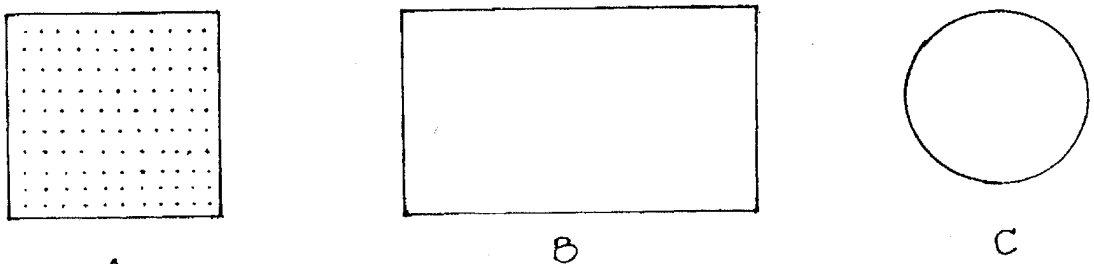

图 3